

Molecular Ecology Laboratory

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Laboratory Protocol

Protocol number: 2
Protocol description: Gender Amp. For all Cetaceans Except Sperm Whale
Original reference: Fain & LeMay
Original entry: Carrie LeDuc
Last updated: 23 October 23, 2003
Updated by: Carrie LeDuc

Required materials:

Nitrile gloves

Primers:

Zfy/x gene:	P15EZ	5' ATA ATC ACA TGG AGA GCC ACA AGC T 3'
	P23EZ	5' GCA CTT CTT TGG TAT CTG AGA AAG T 3'
SRY gene:	Sry Y53-3c	5' CCC ATG AAC GCA TTC ATT GTG TGG 3'
	Sry Y53-3d	5' ATT TTA GCC TTC CGA CGA GGT CGA TA 3'

Controls:

1. A known male and female. These must be strandings or other samplings of your species where sex is known – gonads measured or brought back. If this is not possible, use the closest species and run more than one of each sex.
2. A negative, no template control.

Additional reagents

mQH₂O
10XPCR Buffer (15mM MgCl₂)
dNTPs
Taq
DNA
1X TBE buffer
2% Agarose in TBE
EtBr

Required equipment:

1. PCR machine
2. Agarose gel rig and power supply

Safety:

EtBR is a mutagen that will penetrate latex gloves. Wear nitrile gloves when performing gel procedure.

Procedure:

1. Set up all PCR reactions in a designated pre PCR area
2. Wipe down area and pipets with 10% bleach followed with 70% EtOH
3. Prepare mix for an additional 10% volume or raise number of samples to make extra
4. Include controls as specified above

Mix for one reaction:

<u>Reagent</u>	<u>Concentration</u>	<u>Volume in ul</u>
mQH ₂ O		16.85
10XPCR Buffer	20mM MgCl ₂	2.5
dNTPs	10mM (2.5 ea)	1.5
P15EZ	10uM	0.75
P23EZ	10uM	0.75
Sry Y53-3c	10uM	0.75
Sry Y53-3d	10uM	0.75
Taq	5 unit/ul	0.25
DNA	~100ng/ul	1.0

Make enough mix for your samples, controls, and ~10% extra

Cycling Parameters:

- * 94C 3min
- * 35 cycles of:
 - 94C 0:45
 - 60C 0:45
 - 72C 1:00
- * 72C 5:00

Visualization:

2% Agarose Gel
100 bp ladder
10 ul of PCR product
1-3 ul 6X loading buffer (OrangeG or BromoBlue)
Run at 85 Volts for 30 min.

Determining Gender:

The Zfy/x band will appear between 400 and 500 bp. – both sexes.

The male/SRY band will appear at 200 bp. – males only.

Use your controls to compare/call your samples.

Do not call any ambiguous samples.

Reamplify a random 10% of each batch for replication along with any which were ambiguous on the initial amplification.